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BY

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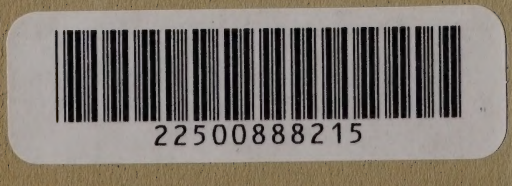

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"The Transmission of the *Trypanosoma Evansi* by Horse Flies, and other Experiments pointing to the Probable Identity of Surra of India and Nagana or Tsetse-fly Disease of Africa." By LEONARD ROGERS, M.D., M.R.C.P., Indian Medical Service. Communicated by Major D. BRUCE, R.A.M.C., F.R.S. Received January 28,—Read February 14, 1901.

(Communicated to the Tsetse-fly Committee of the Royal Society.)

The close resemblance between surra of India and tsetse-fly disease of Africa has long been known, while Koch, after having seen the living *Trypanosoma Evansi* at Muktesar in India, and soon after studied the parallel disease in German East Africa, pronounces them to be the same, and in his 'Reiseberichte' calls the disease seen in the latter place "Surrakrankheit." The appearance of the report made to the Tsetse-fly Committee of the Royal Society by Kanthack, Durham, and Blandford on their experimental investigation of the latter disease, suggested to me to repeat some of their experiments in the case of

surra, with a view to contributing towards the solution of the question of the identity or otherwise of the two diseases, and the following is a brief account of the results obtained while I was in charge of the Imperial Bacteriological Laboratory at Muktesar, during the absence of Dr. Lingard on sick leave.

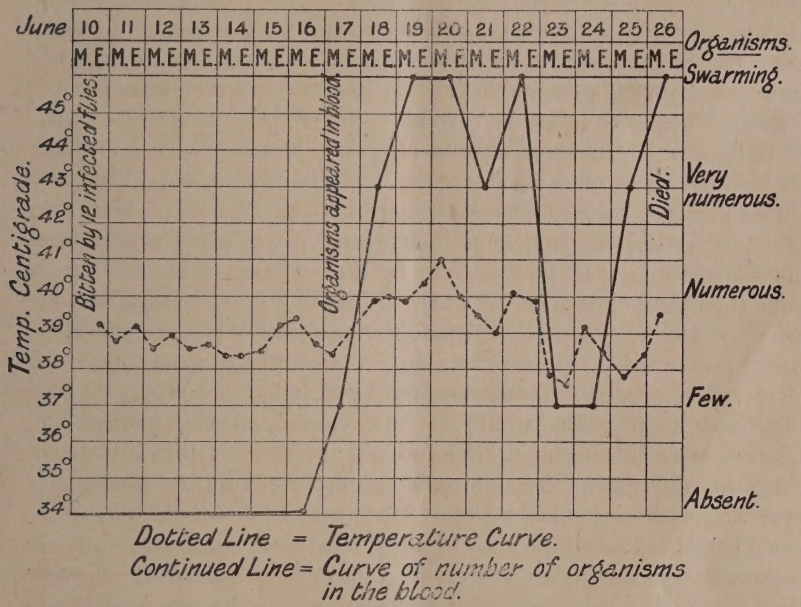
I. *The Transmission of Surra by the Bites of Horse Flies.*

It was proved some years ago by Bruce that the *Trypanosoma Brucei* is carried from one animal to another by the bites of the tsetse fly. As surra can be certainly produced in susceptible animals by the application of infected blood to the smallest scratch in the skin of another susceptible animal, it appeared to be likely that horse flies might carry the infection from one animal to another. A series of experiments were carried out to test this possibility with the following results. Horse flies were caught and kept for varying periods of time after having been allowed to bite and suck the blood of an animal which was suffering from surra, and whose blood at the time contained the *Trypanosoma Evansi* in considerable or large numbers. They were subsequently allowed to bite a healthy animal, dogs and rabbits being used in the experiments, and the former were kept in a different house at some distance from the infected animals, and the latter in separate cages during the incubation period. In every case in which the flies had been kept from one to four or more days after biting the infected animals, no disease ensued in the healthy ones. Many such flies were dissected and microscopically examined, but in no case was anything which might be taken for a development of the trypanosoma in the tissues of the insect detected. A rat was also fed on a number of flies, which had bitten infected animals at varying periods previously, but no infection was thus produced.

When, however, flies which had just sucked infected blood were immediately allowed to bite another healthy animal, positive results were obtained after an incubation period corresponding with that of the disease produced when a minimal dose of infected blood is inoculated into an animal of the same species. The result was uncertain if only one or two flies were allowed to bite, and especially if they were allowed to suck as much blood as they wished without being disturbed. If, on the other hand, several flies, which had just sucked an infected animal, were induced to bite a healthy one, and especially if they were disturbed and allowed to bite again several times, infection was always readily produced in both rabbits and dogs, the fur of the latter having been carefully cut, without abrading the skin, at the site over which the flies were applied. The following is the chart of a typical experiment of this kind. The dog was bitten by twelve flies which had just previously sucked blood from a dog, which was swarming with the

Trypanosoma Evansi, and which had itself been previously infected by the bites of flies experimentally. On the seventh day the organisms were found in the blood in small numbers, and steadily increased during the next two days to swarming—that is, over fifty in the field of a Zeiss D lens, and after oscillations the animal died on the tenth day after the appearance of the organisms in the blood. Post-mortem the usual lesions were found, the spleen being very much enlarged. The right axillary glands were much enlarged, and contained the organisms, while those of the left axilla were but half the size of those

Chart of dog infected by the bites of horse flies which had just previously bitten a surra dog.



of the right side, which is of importance in connection with the fact that the flies had been applied to the upper part of the right side of the body within the area whose lymphatics pass to the right axillary glands. The glands of the right groin were also larger than those of the left, and also contained the organisms in large numbers.

Unfortunately these experiments could not be extended to horses on account of the necessary flies only being found at the height of the Muktesar Laboratory (7800 feet above sea level) during the three or four hottest months, and they were not available in the rainy season when a horse had been obtained for the experiment. The skin of this animal, however, is so thin that it would be likely to be at least as easily infected as a dog, while the facts above recorded will readily

explain the slow and irregular spread of surra through a stable of horses, by the occasional occurrence of the event of a fly which has bitten a diseased animal being disturbed and immediately going off to bite another healthy one. Further, the proof that infection may take place through flies, brings surra into closer resemblance to tsetse-fly disease, and increases the probability of the two being identical, or, at least, caused by very closely allied species of the same family of parasite.

II. *Latent Cases of Surra in Cattle as a Possible Source of Infection.*

Bruce has shown that the parasite of tsetse-fly disease may be present in the blood of big game animals without causing acute symptoms or definite sign of disease, and that their blood when inoculated into susceptible animals will produce the typical acute affection; and further that a very protracted form of the disease may occur in sheep and goats, and possibly form a source of infection for animals. Lingard, in his first volume on "Surra," records the case of a bull which he inoculated with surra, and in whose blood the trypanosoma was found for three days only, shortly afterwards, yet guinea-pigs inoculated with the blood of this bull on the 85th and 163rd days after the first appearance of the parasite developed fatal surra with numerous trypanosoma in their blood. Further inoculations from the bull on the 234th and 267th day proved negative. He has also recorded two naturally acquired cases of the surra in cattle, which proved fatal. These facts suggest the possibility of the latent disease in cattle acting as a source from which biting flies might carry the disease to horses, especially as surra is so frequently met with on the roads to hill stations in India, where numbers of bullock carts are going up and down. It seemed advisable, therefore, to repeat this observation on surra in cattle, so I inoculated a small hill bull intravenously with a small quantity of blood from a rabbit, which contained numerous trypanosoma. The result confirmed Dr. Lingard's observation, for on the seventh day after inoculation the organism appeared in small numbers in the blood of the bull, remained present for four days, and subsequently was not detected during the next 161 days of the disease, while the animal, after showing slight signs of illness for about a month, remained subsequently in apparently good health, except for an occasional slight rise of temperature for two or three days. A rat, which was inoculated on the 30th day of the disease, and two rabbits inoculated on the 59th and 141st days respectively, developed fatal surra, with large numbers of the trypanosoma in their blood; that on the latest-mentioned date having been done during a temporary rise of temperature of the bull without the presence of any trypanosoma.*

* All the rats used in experiments mentioned in this paper had been first proved to be free from the *Trypanosoma sanguinis*, except where otherwise stated.

However, the incubation period was an unusually long one, namely, fifteen days, against from four to six days in the case of rabbits inoculated with the blood of a surra animal which contained the trypanosoma. My observations on intermediate developmental forms of the trypanosoma are not sufficiently advanced for any definite statement on the forms present in the bull's blood at the time these inoculations were made.

A very similar result was obtained in the case of a sheep, in which the trypanosoma appeared seven days after inoculation with the blood of a surra dog, remained present for six days in small numbers, and was then absent for thirty days, during which the animal showed definite symptoms of somewhat mild surra, but improved somewhat latterly. At this period it was handed over to Dr. Lingard, on his resuming charge of the Muktesar Laboratory, and I am unable to give the final result as he has not acceded to my request for information on the point. A goat inoculated at the same time showed the surra organism in its blood on the fourth day, and continued to show it at intervals up to the twenty-sixth day, after which it was absent for the remaining thirteen days that it was under my observation; but this animal was much more ill than the sheep, and became greatly wasted, and presented œdematous swellings on the legs, enlargement of the lymphatic glands, yellow marks on the conjunctiva, and nasal discharge. Lingard also records one case in a sheep which was fatal after 127 days, and three experiments on goats in which the disease was fatal on from the 58th to the 186th day.

In all three animals, then, surra tends to run a prolonged and chronic course, and especially in the case of cattle and sheep; in the latter of which surra affords an additional point of resemblance with tsetse of Africa. It has been thought by some that the difference in the course of the two diseases in the case of cattle is a strong argument against surra and tsetse-fly disease being identical, as the latter is a much more fatal disease in these animals than surra is in India. The difference, however, is but one of degree, for cattle in South Africa not unfrequently do recover from the disease of that country, while surra may be fatal to cattle in India, and may, indeed, prove to be much more frequently so than is at present imagined, when diseases of cattle are more closely studied in India than they have as yet been. Further, Koch has recently shown that the disease in German East Africa is absolutely fatal to the ordinary breeds of donkeys in that country, yet the Masai donkeys are absolutely immune. This shows a difference of susceptibility between different breeds of the same animal to the same (African) disease, much greater than that existing between two breeds of cattle in South Africa and India respectively towards the two diseases nagana and surra. Hence this argument against the identity of the two affections loses much, if not all, its weight. The

possibility of latent forms of surra in cattle, and possibly also in sheep and goats, in India taking the place of similar infections in wild game in the case of tsetse-fly disease in South Africa is, then, worthy of consideration, and the two may be closely analogous.

III. *Feeding Experiments.*

Kanthack, Durham, and Blandford record that they were unsuccessful in most of their experiments in producing infection of Nagana, by feeding animals on material containing the organism of the disease, the possibility of infection appearing to depend on accidental lesions of the nose and mouth, &c. Lingard, on the contrary, records in his first volume on "Surra" one negative result in a horse after the ingestion of 200 c.c. of infected blood, and one positive one 75 days after the last, and 130 after the first, dose of blood by the mouth, small quantities of material being given at frequent intervals. As he was working in an infected district, and the incubation period was an extraordinarily long one, this experiment can hardly be accepted as conclusive, especially in view of the proof given above, that the disease can be carried by flies. That spontaneous infection did occur in some way in the course of his experiments is clear from the case which he records, in which a horse, which was being given large doses of arsenic as a prophylactic measure, spontaneously developed the disease before he was inoculated, very possibly through infection by flies from some other animal under experiment. This possible source of fallacy is excluded in the few experiments I have carried out on this point, by the fact that they were performed at a time of the year when there were no biting flies to be found. With the exception of one rabbit, which was fed on $\frac{1}{2}$ c.c. of surra blood swarming with the organism, in 10 c.c. of milk, with a negative result, rats were used in these experiments, either some organ of an animal dead of surra, or the blood of the same in milk being given. At first the results, although usually negative, were not always so, as in the case of Kanthack's experiments. A possible source of infection was found in the fact that some of the animals had previously been examined for the *Trypanosoma sanguinis* the same morning as the feeding experiment was carried out, and one of the animals was observed to lick the wound in its tail in the intervals of feeding on the infected material. This source of infection was then carefully excluded, and several experiments were done in which a little surra blood in milk was given to two rats, one of which was untouched, while in the case of the other the nose and mouth were first abraded. In each case the untouched rat escaped infection, while the one with abrasions contracted fatal surra after the usual incubation period for the inoculated disease. These experiments, then, support the view that infection in the case of feeding is through some lesion in the skin or mucous

membranes, and once more the results obtained in the case of surra are precisely similar to those got in the researches on tsetse-fly disease conducted under the Committee of the Royal Society.

IV. *Is the* Trypanosoma sanguinis *related to Surra?*

It is pretty generally agreed that the *Trypanosoma sanguinis* of rats is distinct, both morphologically and pathologically, from nagana and surra, although in the case of the latter disease Dr. Lingard claims to have produced surra in horses and other animals by inoculating this organism. The incubation period, however, in his four successful out of twelve experiments in horses, varied between 7 and 65 days, although on the next passage it returned at once to the ordinary period for surra of about 7 days. This remarkable fact, taken in conjunction with his having worked in an infected area, and with the proof of the possibility of flies carrying the disease, makes it possible that the infection was produced by some other agency than the rat's parasites. I recently inoculated a pony intravenously with 2 c.c. of the blood of a rat infected with the *Trypanosoma sanguinis*, with a negative result during the 55 days it was under my observation, the blood being examined daily, the experiment having being carried out at a time of the year when no biting flies were to be found, and in a non-endemic area. It may thus be worthy of record in this connection, as although but an isolated one, it is in agreement with the results of Vandyke later.

Another pony inoculated with a few drops of the blood of a surra dog five days after the one just mentioned, developed surra on the ninth day, as shown by the presence of the *Trypanosoma Evansi* in its blood. A negative result was also obtained in the case of a dog which was twice inoculated with the *Trypanosoma sanguinis* and examined daily for 82 days.

Rats, which had been found to harbour the *Trypanosoma sanguinis*, were also inoculated with surra, and after the usual incubation period in these animals of about four days the *Trypanosoma Evansi* appeared in the blood, and were easily distinguished from the former parasite by their much shorter and blunter ends. They increased daily until in most of the cases over 50 were present in a field of a Zeiss D lens, while the original rat organisms remained at about the same numbers as before the inoculation with the surra blood. The two organisms, therefore, appear to me to be quite distinct both morphologically and pathologically.

In every point, then, that I have so far investigated, the results obtained in the case of surra closely agree with those of the Royal Society's Committee in tsetse-fly disease, and so far as they go they support the view that the two diseases are probably identical. I had

hoped to have been able to make arrangements for studying both diseases side by side, but have not yet been able to do so on account of the disturbed state of South Africa.

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